50. A method according to Claim 37 for identification of a cell surface or secreted protein.

REMARKS

Claims 1-27, 29-40, and 42-50 are now pending. Applicants have amended claims to delete multiple dependencies and to correct minor typographical and grammatical errors. Claims 1 and 17 are amended to recite an *in vitro* method of expressing a DNA in a pluripotent cell. This amendment is supported by the specification as filed, for example, at page 12, first paragraph and page 34, first paragraph. Claim 1 has been further amended to recite that two DNA's are expressed from the second vector, the first DNA encoding a selectable marker and the second DNA encoding a product that is not a selectable marker. Support for this amendment is supported by the specification as filed, for example, at pages 2-3, 8 and 20.

Applicants have amended claim 5 and 27 to recite that the selectable marker is an antibiotic resistance gene. This amendment is supported by the specification as filed, for example, at page 6.

Claims 33 and 37 are amended to identify the cell type as pluripotent. Claim 37 is further amended to by replacement of the term "capable of directing" with the term "that directs." The subject matter of claim 41 has been added to claim 37 and claim 41 has been canceled.

Claims 1, 6, 17, 33, 37, and 47 are amended to correct a spelling error. Claims 11 and 23 are amended to delete the exemplaray subject matter and claim 20 is amended to delete the term "substantially."

None of the claim amendments adds new matter.

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In view of the foregoing amendments and remarks, applicants respectfully request the examination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this amendment and charge any additional required fees to deposit account 06-0916.

Respectfully submitted,

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APPENDIX OF AMENDED CLAIMS

1. [A] An in vitro method of expressing a DNA in a pluripotent cell, comprising:

(a) (i) transfecting the cell with a first vector that expresses a replication factor;

or

(ii) otherwise obtaining a cell that expresses or will express the replication factor;

and

(b) transfecting the cell with a second vector, wherein

(i) the second vector contains a DNA [,or is adapted to receive a DNA,] coding for a selectable marker in operative combination with a promoter for expression of the [DNA] selectable marker;

[and]

(ii) the second vector additionally contains a second DNA in operative combination with a promoter for expression of the second DNA, and which second DNA does not code for a selectable marker; and

[(ii)] (iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor.

2. A method according to Claim 1 wherein the replication factor is a viral replication factor.

3. A method according to Claim [1 or] 2 wherein the viral replication factor is selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof appropriate to the cell species.

4. A method according to [any of] Claim[s] 1[-3] wherein the second vector does not express the replication factor.

5. A method according to [any of] Claim[s] 1[-4] wherein the [second vector expresses a] selectable marker is an antibiotic resistance gene.

6. A method according to [any of] Claim[s] 1 [-5] further comprising transfecting the cell with a third vector, wherein the third vector contains a DNA, or is adapted to receive a DNA, in operative combination with a promoter for expression of the DNA, and replication of the third vector is dependent upon presence within the cell of the replication factor.

7. A method according to Claim 6 wherein the third vector expresses a selectable marker, which selectable marker is different to that expressed by the second vector.

8. A method according to [any preceding claim] Claim 1 wherein the cell is selected from the group consisting of a mammalian cell [or] and an avian cell.

9. A method according to [any preceding claim] Claim 1 wherein the cell is an embryonic cell.

10. A method according to Claim 9 wherein the cell is an ES cell.

11. A method according to [any preceding claim] Claim 1 for transfection of an ES cell wherein the ES cell of step (a) expresses polyoma large T antigen and the second vector comprises a natural target for polyoma large T antigen. [such as *Ori* or functional variants thereof adapted to bind to polyoma large T antigen.]

12. A method according to [any preceding claim] Claim 1 wherein the DNA codes for a polypeptide or protein.

13. A method according to [any of] Claim[s] 1 [-11] wherein the DNA codes for an antisense RNA.

14. A method according to Claim 1 [any preceding claims] wherein the promoter is inducible.

15 A method according to [any preceding claim] Claim 1 wherein transcription of the DNA can be activated by a site specific recombinase.

16. A method according to [any preceding claim] Claim 1 wherein replication of the second vector can be prevented by a site specific recombinase.

17. A vector for transfection of a pluripotent cell in vitro, wherein:

(i) the vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker;

(ii) the vector contains a second DNA in operative combination with a promoter for expression of the DNA, and which second DNA does not code for a selectable marker;

(iii) extrachromosomal replication of the vector is dependent upon presence within the cell of a replication factor; and

([iii] iv) the vector does not express the replication factor.

18. A vector according to Claim 17 wherein the replication factor is a viral replication factor.

19. A vector according to Claim [17 or] 18 wherein the viral replication factor is selected from the group consisting of polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof.

20 A vector according to [any of Claims 17 to 19] Claim 17 wherein the vector is [substantially] free of DNA coding for the replication factor or any part thereof.

21. A vector according to [any of Claims 17 to 20] Claim 17 for transfection of mammalian or avian cells.

22 A vector according to [any of Claims 17 to 21] Claim 17 for transfection of ES cells.

23 A vector according to Claim 22 comprising a natural target for polyoma large T antigen [, such as *Ori* or functional variants thereof adapted to bind to polyoma large T antigen].

24 A vector according to [any of Claims 17-23] Claim 17 wherein the DNA codes for a polypeptide or protein.

25 A vector according to [any of Claims 17-23] Claim 17 wherein the DNA codes for an antisense DNA.

26 A vector according to [any of Claims 17-25] Claim 17 wherein the promoter is inducible.

27. A vector according to any [of Claims 17 to 26] Claim 17 wherein the [vector comprises a sequence coding for a] selectable marker is an antibiotic resistance gene.

~~28. [Use of a vector according to any of Claims 17-27 for expression of a DNA sequence within a cell.]~~

29 An ES, EC or EG cell transfected with a first vector that expresses a replication factor and with a second vector according to [any of Claims 17 to 27] Claim 17.

30. A mammalian cell according to Claim 29.

31. An embryonic cell according to Claim 29.

32. A cell selected from an ES, EC or EG cell according to [any of] Claim[s] 29 [to 31], and differentiated progeny thereof.

33. An assay for the effect of presence in a pluripotent cell of a protein or polypeptide or other product of DNA expression, comprising the steps:

(a) (i) transfecting the cell with a first vector that expresses a replication factor; or

(ii) otherwise obtaining a cell that expresses or will express the replication factor;

(b) transfecting the cell with a second vector, wherein

(i) the second vector contains a DNA coding for the protein or polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA;

(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

(iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor;

(c) selecting for cells that have been transfected with the second vector; and

(d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the protein or polypeptide or other product of DNA expression.

34. An assay according to Claim 33 wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate assays in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

35. An assay according to Claim 33 [or 34] for assay of the effect of presence in the cell of two factors, each factor being independently selected from a protein, a polypeptide and another product of DNA expression.

36. A method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the method of [any of] Claim[s] 33 [to 35].

37. A method of investigating the properties of a DNA sequence comprising expressing in a pluripotent cell a composite DNA including (a) the DNA sequence under investigation, linked to (b) a DNA coding for a cell active protein, wherein

(i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface, [and]

(ii) the DNA of (b) does not code for a polypeptide [capable of] that direct[ing]s transportation of the cell active protein to the cell surface, and

(iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.

38. A method according to Claim 37 for screening a library of DNAs to identify DNA sequences coding for signal polypeptide sequences that transport proteins to the cell surface, and the method optionally comprises determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.

39. A method according to Claim 37 [or 38] wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transportation of the protein to the cell surface.

40. A method according to [any of Claims 37 to 39] Claim 37 wherein the cell active protein induces a morphological or proliferative change in the cell.

~~41. [A method according to any of Claims 37 to 40 wherein the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.]~~

42. A method according to [any of Claims 37 to 41] Claim 37 wherein the cell active protein is a cell surface receptor.

43. A method according to Claim 42 wherein the cell active protein is an IL-6 receptor and the DNA of (b) encodes a modified form of the receptor preprotein lacking a functional signal sequence.

44. A method according to [any of Claims 37 to 43] Claim 37 comprising investigating the properties of a DNA in mammalian or avian cells.

45. A method according to [any of Claims 37 to 44] Claim 37 comprising investigating the properties of a DNA in embryonic cells.

46. A method according to Claim 45 comprising investigating the properties of a DNA in ES, EC or EG cells or differentiated progeny of such cells.

47. A method according to [any of Claims 37 to 46] Claim 37 comprising expressing the composite DNA by :

(a) (i) transfecting the cell with a first vector that expresses a replication factor; or

(ii) otherwise obtaining a cell that expresses or will express the replication factor;

(b) transfecting the cell with a second vector, wherein

(i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;

(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

(iii) extrachromosomal replication of the second vector is dependant upon presence within the cell of the replication factor;

(c) selecting for cells that have been transfected with the second vector; and

(d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the composite DNA.

48. A method according to [any of Claims 37-46] Claim 37 wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

49. A method according to [any of Claims 37 to 47] Claim 37 for identification of a DNA coding for a cell surface or secreted protein.

50. A method according to [any of Claims 37 to 47] Claim 37 for identification of a cell surface or secreted protein.